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*Mamu-B*08*-Positive Macaques Control Simian Immunodeficiency Virus Replication▽

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Certain major histocompatibility complex (MHC) class I alleles are associated with the control of human immunodeficiency virus and simian immunodeficiency virus (SIV) replication. We have designed sequence-specific primers for detection of the rhesus macaque MHC class I allele *Mamu-B*08* by PCR and screened a cohort of SIV-infected macaques for this allele. Analysis of 196 SIV_{mac}239-infected Indian rhesus macaques revealed that *Mamu-B*08* was significantly overrepresented in elite controllers; 38% of elite controllers were *Mamu-B*08* positive compared to 3% of progressors ($P = 0.00001$). *Mamu-B*08* was also associated with a 7.34-fold decrease in chronic phase viremia ($P = 0.002$). *Mamu-B*08*-positive macaques may, therefore, provide a good model to understand the correlates of MHC class I allele-associated immune protection and viral containment in human elite controllers.

Certain HLA class I alleles strongly influence whether individuals become slow or rapid progressors after human immunodeficiency virus (HIV) infection (8, 18–20, 28, 30, 32, 39). Long-term nonprogressor/elite controller (EC) cohorts are enriched for *HLA-B27* and *HLA-B57*. Numerous studies have implicated these molecules in the presentation of epitopes that elicit effective HIV-specific $CD8^+$ T-lymphocyte responses (2, 3, 13, 17, 21, 22, 24, 31, 32). Unfortunately, there are many difficulties associated with understanding the basis for these HLA associations in HIV-infected people, including inoculum variability, allelic diversity, and access to acute-phase samples. Studying simian immunodeficiency virus (SIV)-infected rhesus macaques that control viral replication may shed some light on why certain *HLA-B27*- and *HLA-B57*-positive humans control HIV infection.

Although certain major histocompatibility complex (MHC) class I alleles, including *Mamu-A*01* and *Mamu-B*17*, are associated with slow disease progression in SIV-infected macaques (33–35, 38, 41, 42), independently, the presence of these alleles is not predictive for disease outcome. In the case of *Mamu-B*17*, only one-fourth of *Mamu-B*17*-positive macaques become ECs, controlling SIV replication below 1,000 viral RNA (vRNA) copies/ml of plasma during the chronic phase of SIV infection (41). Even *Mamu-B*17*-positive macaques containing identical MHC class I haplotypes have widely divergent disease courses (40).

Recently, we defined novel Vif- and Nef-specific $CD8^+$ T-cell responses in three EC macaques. We discovered that these epitopes were recognized by T cells restricted by the MHC class I molecule, Mamu-B*08 (J. T. Loffredo et al., unpublished data). Therefore, we investigated the impact of *Mamu-B*08* on SIV disease progression in a cohort of 196 SIV_{mac}239-infected Indian rhesus macaques.

Development of PCR-SSP for *Mamu-B*08*. To identify *Mamu-B*08*-positive animals, rhesus macaques (*Macaca mulatta*) of Indian descent were genotyped for the MHC class I allele, *Mamu-B*08*, using PCR amplification with sequence-specific DNA priming (PCR-SSP) methodology as previously described (27, 36). The nucleotide sequences of the primers used for typing *Mamu-B*08* were as follows: forward, 5′-CGT GAG GCG GAG CAG GTC-3′; and reverse, 5′-CCA CAG CTC CGA TGA ACA CAG-3′. Because *Mamu-B*08* and *Mamu-B*03* are strikingly similar in the regions encoding the alpha-1 to alpha-3 domains (6), we designed the reverse primer for the *Mamu-B*08* PCR-SSP reaction to target multiple unique polymorphisms present in the exon encoding the transmembrane region of *Mamu-B*08* (Fig. 1A and B). Primers were diluted to a working concentration of 1 μM.

Thermal cycling conditions were identical to methods previously published (27), with the exception of the second annealing temperature being 67.9°C for 21 cycles. At the completion of 30 thermal cycles, the PCR underwent a final extension at 72°C for 8 min, followed by a terminal hold at 25°C. Subsequently, PCR products were electrophoresed on 2% agarose gels at a constant voltage in 0.5× sodium borate buffer (7). The corresponding *Mamu-B*08* allele-specific amplicon was ~1,096 bp (Fig. 1C). Each *Mamu-B*08* typing reaction also included primers to target an ~300-bp fragment of the class II *Mamu-DRB* as an internal control (23). MHC class I typing reactions were not considered valid without the presence of this internal control amplification product. Amplicon length was measured relative to a 100-bp DNA ladder (Invitrogen, Carlsbad, CA). Amplification specificity of the *Mamu-*

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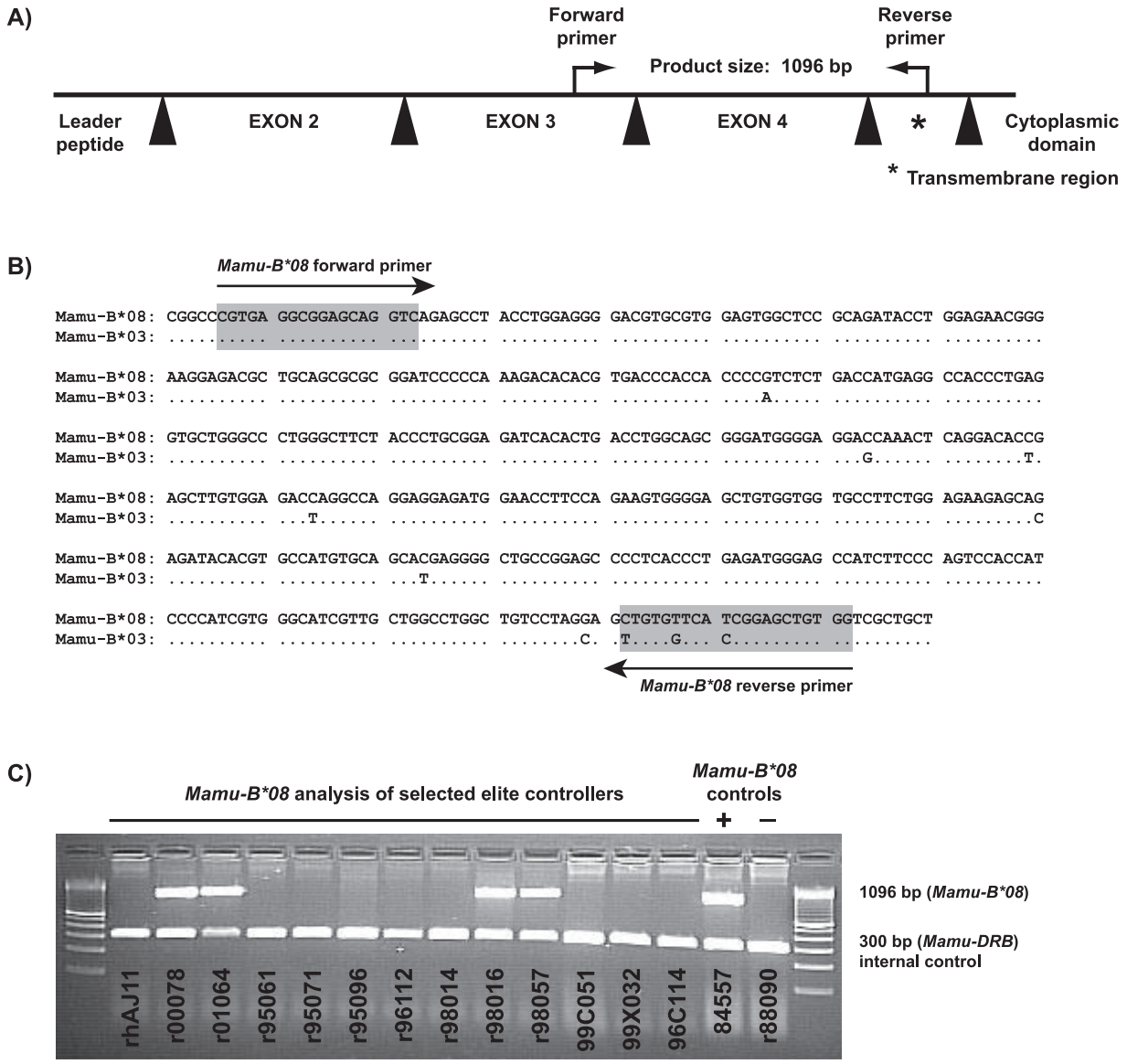

FIG. 1. Design of PCR-sequence specific primers (PCR-SSP) to amplify the MHC class I allele *Mamu-B*08* from genomic DNA. (A) Primers were designed to amplify a 1,096-bp product that contains part of exon 3 (77 bp), intron 3 (573 bp), exon 4 (276 bp), intron 4 (106), and 64 bp of the transmembrane region (exon 5) of *Mamu-B*08*. Black triangles represent *Mamu-B*08* introns. (B) Sequence similarity between *Mamu-B*08* and *Mamu-B*03*. To avoid amplification of *Mamu-B*03*, the *Mamu-B*08* primers were designed such that the forward primer amplifies *Mamu-B*08* or *Mamu-B*03* and the reverse primer takes advantage of three unique polymorphisms in the transmembrane region (exon 5), allowing for specific detection of *Mamu-B*08* only. (C) PCR-SSP genotyping of *Mamu-B*08* from genomic DNA of selected elite controller macaques. Macaques 84557 and r88090 were used as *Mamu-B*08* positive and negative controls, respectively. Previously, a cDNA library isolated from 84557 indicated that this macaque expresses *Mamu-B*08*. *Mamu-B*08* was not found in a cDNA library from macaque r88090 (6). PCR-SSP primers that amplify a portion of *Mamu-DRB* (~300 bp) were included as an internal control to minimize false negatives. A 100-bp DNA ladder was used to interpret amplicon sizes.

*B*08* typing primers was confirmed by using previously established sequencing methods (18a) in 44 *Mamu-B*08*-positive Indian rhesus macaques.

The remaining MHC class I alleles (*Mamu-A*01*, *-A*02*, *-A*08*, *-A*11*, *-B*01*, *-B*03*, *-B*04*, *-B*17*, and *-B*29*) were typed as previously described (18a, 27).

Colony frequencies of *Mamu-B*08*. After completion of PCR-SSP development, we screened eight different Indian rhesus macaque colonies to determine the frequency of *Mamu-B*08* (Table 1). After testing >2,900 macaques, we found that the overall frequency of *Mamu-B*08* was ~5.8%. At the Wisconsin National Primate Research Center (WNPRC), the frequency of *Mamu-B*08* was 6.8% (1,271 macaques tested). Overall, the frequency ranged from 0.9 to 14.1% across the various macaque colonies.

***Mamu-B*08* is significantly enriched in a cohort of elite controller macaques and is associated with lower viremia in the chronic phase of SIV infection.** We next investigated the frequency of *Mamu-B*08* in a cohort of previously identified EC macaques (41). Four of the thirteen ECs expressed *Mamu-*

TABLE 1. Frequency of *Mamu-B*08* in different Indian rhesus macaque colonies

Colony[a]	No. of *Mamu-B*08*$^+$ animals	Total no. of animals	Frequency (%)
Southern Research Institute (Birmingham, AL)	1	113	0.9
Yerkes NPRC	3	141	2.1
Various NIH/NCI-funded projects	8	380	2.1
Tulane NPRC	3	122	2.5
Caribbean Primate Research Center	18	451	4.0
California NPRC	11	163	6.7
Wisconsin NPRC	86	1271	6.8
Oregon NPRC	41	290	14.1
Total	171	2,931	5.8

[a] NPRC, National Primate Research Center; NIH/NCI, National Institutes of Health and National Cancer Institute.

*B*08* (Fig. 1C). Interestingly, three of the four *Mamu-B*17*-negative ECs, r00078, r01064, and r98057, were *Mamu-B*08* positive. After testing a cohort of 192 SIV-infected macaques for *Mamu-B*08*, we identified seven *Mamu-B*08*-positive macaques, four of which were from this initial EC cohort.

To investigate the role of *Mamu-B*08* in control of pathogenic SIV replication, we recently infected four additional *Mamu-B*08*-positive Indian rhesus macaques with $SIV_{mac}239$ (100 50% tissue culture infective doses, intravenously). Two of these *Mamu-B*08*-positive macaques, r00032 and r02019, had viral loads below 1,000 vRNA copies/ml at 22 weeks postinfection. We have included them in Table 2 as ECs due to their low plasma viremia early into the chronic phase of SIV infection. All four recently infected *Mamu-B*08*-positive macaques were included in the statistical analysis.

PROC LOGISTIC regression analysis was performed on 196 SIV-infected Indian rhesus macaques from two independent cohorts (143 animals from D. Watkins' studies and 53 animals from J. Lifson's studies) to determine whether the frequency of *Mamu-B*08* was enriched in EC macaques. The analysis methods were performed as previously described (41), except the program used was SAS 9.1 (SAS Institute, Cary, NC). All macaques were infected with the molecular clone $SIV_{mac}239$, and plasma virus concentrations were monitored as previously described (10, 26) at multiple time points for a minimum of 10 weeks postinfection. Although vaccinated animals are included in the 196 animal cohort, none of these macaques received a vaccine regimen that demonstrated effective and durable control of SIV replication.

Including the two recently infected *Mamu-B*08*-positive EC animals, 16 $SIV_{mac}239$-infected macaques were identified as

TABLE 2. *Mamu-B*08* is significantly overrepresented among elite controller macaques[a]

Animal	Presence (+) or absence (−) of MHC class I allele or value for parameter									
	*A*01*	*A*02*	*A*08*	*A*11*	*B*01*	*B*03*	*B*04*	*B*08*	*B*17*	*B*29*
rhAJ11	−	+	−	+	−	−	−	−	+	+
r00078	−	−	+	−	−	−	−	+	−	+
r01064	−	+	−	−	−	−	−	+	−	−
r95061[b]	+	+	−	−	−	−	−	−	+	+
r95071	−	+	−	−	−	−	−	−	+	+
r95096[c]	+	−	−	+	−	−	−	−	+	+
r96112[d]	−	−	+	−	−	−	−	−	+	+
r98014[e]	−	+	−	+	−	−	−	−	+	+
r98016	−	+	−	−	−	−	−	+	+	+
r98057	−	−	−	+	−	−	−	+	−	−
99C051	−	+	−	−	−	−	−	−	+	+
99x032[f]	−	+	+	−	−	−	−	−	−	−
96C114[g]	+	−	−	−	−	−	−	−	+	+
C59Z[h]	−	−	+	−	−	+	+	−	−	−
r00032	−	+	−	−	−	−	−	+	−	−
r02019	−	−	+	−	−	−	−	+	−	−
Frequency in elite controller cohort (%) (n = 16)[i]	19	**56**	31	**25**	0	6	6	**38**	**56**	**63**
Frequency in entire cohort (%) (n = 196)	38	27	26	7	33	2	2	6	21	22

[a] Sixteen $SIV_{mac}239$-infected Indian rhesus macaques that maintained chronic phase (≥10 weeks) plasma virus concentrations of <1,000 vRNA copies/ml were identified as ECs. This 1,000 vRNA copies/ml EC threshold represents a >2-log reduction in chronic-phase plasma virus concentrations compared to the chronic-phase geometric mean of the entire cohort of 196 SIV-infected macaques (223,800 vRNA copies/ml). Viral control was maintained for >1 year during the chronic phase of SIVmac239 infection or from week 10 until the animal was assigned to another study. All ECs were infected for >39 weeks (9 months) with the exception of r96112 (38 weeks), along with r00032 and r02019 (22 weeks each in an ongoing study). Animals were vaccine naive except where indicated.

[b] DNA/MVA $Gag_{181\text{-}189}$CM9 epitope only. The vaccine results were previously published (1).

[c] Lipopeptide $Gag_{181\text{-}189}$CM9 epitope only (35).

[d] CpG in incomplete Freund adjuvant (41).

[e] Intramuscular injections of 10^7 irradiated PBMC from SIV-naive animal rh2055 (41).

[f] DNA SIV *gag* prime/Ad5 SIV *gag* boost. The vaccine results were previously published (9, 29).

[g] Whole inactivated SIV virions. The vaccine results were previously published (25).

[h] CpG in PBS (J. Lifson, unpublished results).

[i] Significant enrichments of the elite controller cohort for expression of a particular MHC class I allele are indicated in bold. Enrichment of this elite controller group for MHC class I alleles was determined with PROC LOGISTIC in SAS 9.1 (SAS Institute, Cary, NC). *Mamu-A*02,* $P = 0.01$; *Mamu-A*11*, $P = 0.01$; *Mamu-B*08*, $P = 0.00001$; *Mamu-B*17*, $P = 0.001$; *Mamu-B*29*, $P = 0.0003$.

ECs, defined as maintaining a chronic phase (≥10 weeks) plasma viremia geometric mean below 1,000 vRNA copies/ml (Table 2). *Mamu-B*08* was dramatically enriched in this EC group. Thirty-eight percent of this EC cohort expressed *Mamu-B*08* compared to just three percent of the 180 SIV-infected macaques that did not control viral replication to the EC threshold ($P = 0.00001$). Overall, 6 of 11 (~55%) macaques identified as *Mamu-B*08*-positive became ECs. Two of the eleven *Mamu-B*08*-positive macaques also displayed what we refer to as controller status, limiting viral replication to <22,000 viral RNA copies/ml (one log reduction of the cohort's chronic phase geometric mean, 223,800 vRNA copies/ml) for at least 10 weeks in the chronic stage of SIV infection. Hence, a majority of *Mamu-B*08*-positive macaques (8 of 11 [73%]) displayed better-than-typical viral control of pathogenic $SIV_{mac}239$ replication.

Animal C59Z, a *Mamu-B*03*-positive macaque, was also recently identified as 1 of the 16 EC macaques (Table 2). Although *Mamu-B*03* was previously associated with slow SIV disease progression (12), it has been difficult to conduct additional studies with *Mamu-B*03* due to the low frequency of this allele (overall, <1%; 18a). Interestingly, a previous study demonstrated that Mamu-B*03 and HLA-B27 bind peptides with similar motifs (11). Mamu-B*03 and Mamu-B*08 are almost identical in amino acid sequence (6). There are only two amino acid differences between Mamu-B*03 and Mamu-B*08 in regions that influence peptide binding and antigen recognition (5, 16). Both differences reside in the alpha-1 domain (exon two); the alpha-2 domains (exon 3) of Mamu-B*03 and Mamu-B*08 are identical. Therefore, it is likely that Mamu-B*08 also shares this HLA-B27 binding profile.

We analyzed the association between MHC class I alleles in both acute- and chronic-phase viremia for each MHC class I allele in the 196 $SIV_{mac}239$-infected macaque cohort. With the exception of the program used (SAS 9.1; SAS Institute, Cary, NC), the analysis methods were performed as previously described (41). PROC REG was used to estimate the relative log geometric mean acute phase peak (single highest value from weeks 1 to 3 for each macaque) plasma virus concentration. PROC MIXED was used to estimate the relative log geometric mean for chronic-phase (≥10 weeks) plasma viremia. All typed MHC class I alleles, EC status, and vaccine status were included as covariates in the models.

Although none of the MHC class I alleles correlated with changes in peak viremia during the acute phase of SIV infection (Fig. 2A), several MHC class I alleles affected chronic phase viremia (Fig. 2B). The 11 *Mamu-B*08*-positive macaques had a 7.34-fold decrease in geometric mean chronic phase plasma virus concentrations compared to the rest of the cohort ($P = 0.002$). The reduction in the relative log geometric mean of *Mamu-B*08*-positive macaques was similar to that of macaques expressing *Mamu-B*17* (7.93-fold), an allele previously associated with slow disease progression (35, 41).

We identified another *Mamu-B*08*-positive EC macaque (r99006) not included in the 196 SIV-infected animal cohort described in the present study. Animal r99006 was part of a separate study in which *Mamu-A*01*-positive and *Mamu-B*17*-positive macaques were infected with an engineered $CD8^+$ T-cell escape variant virus (14, 15) to prevent the development of immunodominant $CD8^+$ T-cell responses thought to be

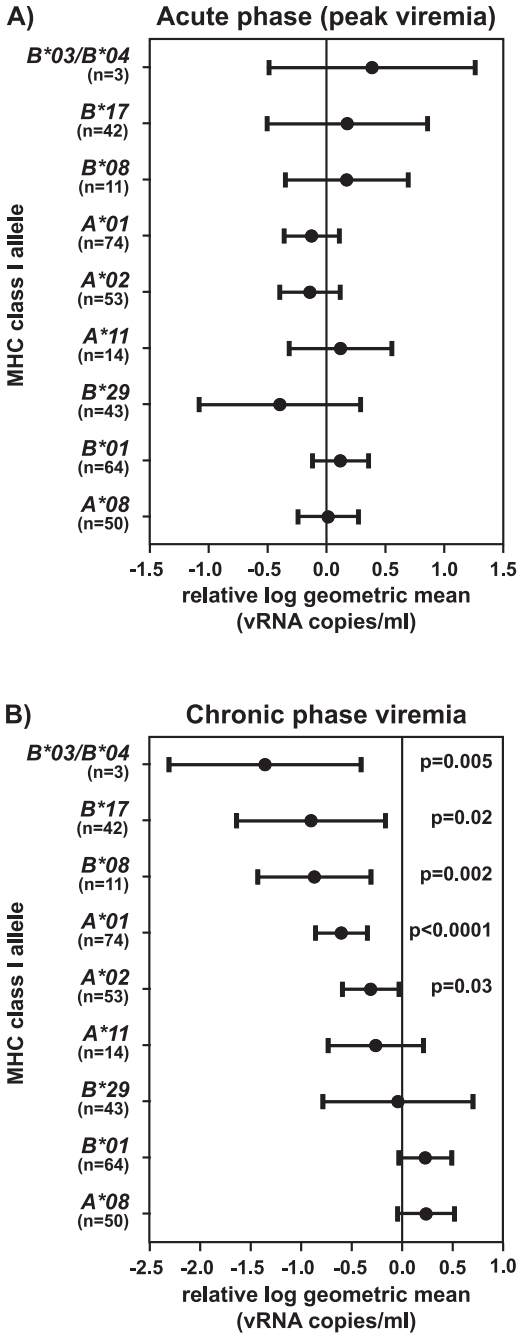

FIG. 2. *Mamu-B*08* influences chronic-phase plasma virus concentrations in $SIV_{mac}239$-infected Indian rhesus macaques. (A) Acute-phase peak viremia (highest plasma virus concentration between weeks 1 and 3 postinfection) is not affected by the presence of the typed MHC class I alleles in 196 $SIV_{mac}239$-infected Indian rhesus macaques. (B) Contribution of typed MHC class I alleles to variation in chronic-phase plasma viremia in the same cohort of 196 macaques. The chronic-phase geometric mean was calculated, and the plasma virus concentration time points ranged from ≥10 weeks to <7.16 years postchallenge. MHC class I effects on SIV plasma viremia are shown with the changes in geometric mean displayed as dots for each MHC class I allele (*Mamu-A*01*, *-A*02*, *-A*08*, *-A*11*, *-B*01*, *-B*03/-B*04*, *-B*08*, *-B*17*, and *-B*29*) relative to the whole cohort and 95% confidence intervals (bars). Probabilities (*P* values) for MHC class I alleles with statistically significant reductions from the relative log geometric mean are displayed. *Mamu-B*03* and *-B*04* were analyzed together because the three *Mamu-B*03*-positive macaques were also positive for *Mamu-B*04*.

associated with viral control (35). Macaque r99006 controlled viremia to <1,000 vRNA copies/ml for almost 4 years after SIV infection before being assigned to another study. We have also identified three additional *Mamu-B*08*-positive macaques from other investigators (D. Evans and G. Franchini, personal communications). At 25 weeks postinfection, two of the three *Mamu-B*08*-positive macaques limited replication of $SIV_{mac}239$ or $SIV_{mac}251$ isolates at or below ~1,000 vRNA copies/ml. In total, 11 of 15 (73%) *Mamu-B*08*-positive macaques controlled replication of pathogenic strains of SIV.

It has been previously reported that *Mamu-B*08* segregates on the same chromosome as *Mamu-B*06* in rhesus macaques (37). Interestingly, when investigating MHC class I cDNA libraries from several *Mamu-B*08*-positive Indian rhesus macaques at the WNPRC, we also noticed that all of these macaques possessed *Mamu-B*06* transcripts (data not shown). None of the cDNA libraries from *Mamu-B*08*-negative macaques possessed *Mamu-B*06* transcripts. However, control of SIV replication is unlikely due to Mamu-B*06. From our studies, Mamu-B*06 does not appear to be involved in the presentation of SIV epitopes (unpublished data). Mamu-B*08, but not Mamu-B*06, was detected by one-dimensional isoelectric focusing (6). This suggests that *Mamu-B*06* may not encode an expressed MHC class I protein.

*Mamu-B*08*-positive Indian rhesus macaques might provide an insightful model for understanding the influence of particular MHC class I alleles on viral control. We have recently identified several Mamu-B*08-restricted $CD8^+$ T-cell epitopes in the chronic phase of SIV infection (Loffredo et al., unpublished). We are currently determining whether $CD8^+$ immune responses restricted by Mamu-B*08 exert strong patterns of immunodominance similar to those associated with *HLA-B27* and *HLA-B57* (2, 4, 22). Mamu-B*08-restricted SIV epitopes should be useful for future pathogenesis studies, including the generation of MHC class I tetramers, to study differences in the $CD8^+$ T-cell responses between progressor and EC *Mamu-B*08*-positive macaques. Finally, given the independent influence of this allele on the outcome of SIV infection, it might be prudent to remove *Mamu-B*08*-positive macaques from vaccine studies.

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